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Expression of vascular endothelial growth factor in the cyst fluid of human cerebral gliomas

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Abstract. Vascular endothelial growth factor (VEGF) or vascular permeability factor (VPF) has been shown to play a key role in angiogenesis in several solid tumours including human brain neoplasms. Its expression has also been found to be correlated to malignancy in the major class of these tumours, gliomas. Moreover, it has been suggested that cyst fluids (CFs) associated with human gliomas may contain a permeability factor responsible for the formation of brain edema and disruption of the blood-brain barrier generally observed in these tumours. We demonstrate that VEGF is present in low and high grade gliomas of seven patients. We also show that VEGF concentration increases with increasing malignancy of the tumours. Although further cases should be investigated, these results suggest that the amount of CF-VEGF may be of value in the diagnosis of human gliomas.

Introduction

A major hallmark of gliomas, the major form of human intrinsic brain tumours, is their striking neovascularization (1). Angiogenesis is essential for proliferation of the primary tumour as well as the secondary foci arising as a result of tumour spread. One factor which has been shown to play a central role in tumour angiogenesis, in general, and in glioma neovascularization, in particular, is the vascular endothelial growth factor, VEGF (2). Indeed, the VEGF gene was originally cloned from human glioblastoma cells as an angiogenic factor homologous to the platelet derived growth factor, PDGF (3). It was also colocalised with another well known angiogenic agent, basic fibroblast growth factor (bFGF), in neoplastic glial cells surrounding blood vessels (2,4,5). VEGF is produced by human glioma cells to act in a paracrine fashion on receptors expressed almost exclusively on endothelial cells of neoformed blood vessels (2,6,7). VEGF specific antibodies inhibited both tumour angiogenesis and tumour growth demonstrating the critical role of this factor in tumour progression *in vivo* (8). This is substantiated by a recent report showing that a dominant-negative mutant of the receptor flk-1 prevented the growth of glioblastoma cells *in vivo* without affecting their growth *in vitro* (9).

VEGF was initially described as a vascular permeability factor (VPF) owing to its capacity to increase capillary vascular permeability after intradermal injection into guinea pigs (10). This experiment suggested that VPF may be responsible for the vasogenic brain edema that is a characteristic of human gliomas and metastatic intracerebral tumours (10). Indeed, the marked neovascularisation of human malignant gliomas is generally associated with cerebral edema, vein thrombosis, and tumour necrosis (1). Occasionally, human gliomas are also associated with formation of a cyst fluid (CF) (1). Although, the composition of CF is not completely known, few reports demonstrated the presence of hormones, cytokines, and growth factors in CFs of human brain tumours. Prisell *et al*, (11) demonstrated the presence of the growth-promoting hormones, somatomedins, in tumour cyst fluid taken from patients with glioma at elevated levels compared with normal serum levels. Active forms of the strong immunosuppressor transforming growth factor beta 2 (TGF-ß2) were also detected in tumour cyst fluid from glioblastoma patients (12). The cyst fluid of human gliomas has also been found to contain interleukin 8 (IL-8) (13), insulin-like growth factor (IGF)-I, -II and IGF-binding proteins (14), and monocyte chemoattractant protein-1 (MCP-1) (15). Interestingly, Whittle *et al*, (16) reported, in a feline infusion model of brain edema, that human glioma cyst fluid infusates increased local white matter water content and caused marked extracellular edema. This was associated with significant endothelial and astrocytic swelling, focal endothelial necrosis, and basement membrane disruption. These results suggested the existence of glioma derived permeability factors in CFs. In this study, we set out to investigate this hypothesis.

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Key words: endothelial growth factor, cyst fluid, cerebral gliomas

Materials and methods

Materials. Vascular endothelial growth factor and antibodies for ELISA assays were from R & D Systems Europe Ltd. (Abingdon, UK). All other chemicals for ELISA assays were obtained from Sigma Chemical (Dorset, UK). 96-well plates were from Marathon LS (London, UK).

TCF-sampling. Human CF samples were obtained by stereotactic aspiration or aspiration through an indwelling catheter at the time of craniotomy for therapeutic and diagnostic purposes. All samples were centrifuged to remove coagulated material, and the supernatants were frozen at -70°C until use.

ELISA assay. The 96-well plates were coated with 50 µl of samples per well for 48 hours at 4°C. The wells were washed 4 times with 300 µl/well of washing buffer (0.123 M NaCl, 0.01 M Na_2HPO_3, 0.05% Tween-20), blocked with 300 µl of 5% BSA in PBS and left at 4°C for 48 h. The plates were washed as above and 100 µl/well of mouse anti-hVEGF monoclonal antibody at a dilution of 1:1000 in PBS was added. Samples were incubated for 4 h at 4°C, then washed and 100 µl/well of biotinylated anti-mouse IgG at 1:1000 in PBS was applied for 1 h at room temperature. After 30 minutes incubation, the plates were washed again and 100 µl of avidin/biotin-horseradish peroxidase was added to each well for 30 minutes at room temperature. The plates were then washed five times and incubated with 100 µl/well of TMB (0.1 mg/ml) and H_2O_2 (1/1000) for 15 minutes at room temperature. The enzymatic reaction was stopped by adding 100 µl/well of 10% H_2SO_4. The amount of VEGF was quantitatively determined by the absorbance measured (at 570 nm) with an ELISA-plate reader. Concentrations of VEGF in test samples were determined by comparing their specific absorbance with those obtained for the standards plotted on a standard curve. Each assay was set in twelve replicates and repeated at least three times.

Results and Discussion

This study was carried out on samples taken from seven patients with cerebral gliomas. The results are summarized in Table I. Two of these patients, 35- and 30-year old, were diagnosed with grade II astrocytomas at the temporal lobe. VEGF was found to be present at 0.051 and 0.068 µg/ml in the CF of these two patients, respectively. Three patients; 43-year old, 37-year old, and 63-year old were diagnosed with grade III astrocytomas at the temporal lobe, the parietal lobe, and temporal lobe, respectively. The cyst fluid of these patients contained 0.098, 0.145, and 0.260 µg/ml of VEGF. It is interesting to note that the 37-year old patient was previously (1987) diagnosed with a grade II astrocytoma and underwent both radiotherapy and chemotherapy before recurrence with a grade III astrocytoma. The amount of VEGF in the CF of this patient was two times more than in grade II astrocytomas. The CFs of two patients (64- and 56-year old), diagnosed with glioblastoma multiforme at the temporal lobe and the parietal lobe, were found to contain 10 to 18 times more VEGF than the grade II astrocytomas, and 2.6 to 10 times more than grade III astrocytomas. Although a more significant number of human gliomas has to be investigated, the data presented show that VEGF is indeed expressed in CF and may, therefore, explain the CF-induced edema and blood-brain barrier disruption observed in animal models (16). The results presented also suggest that the amount of CF-VEGF is correlated to the grade of malignancy; VEGF amounts being increased with increasing malignancy. This result is in agreement with the pattern of VEGF expression in human gliomas. Indeed, Plate *et al*, (2) demonstrated that VEGF is induced in astrocytoma cells but is dramatically upregulated in glioblastoma cells. In addition, they found that the high-affinity tyrosine kinase receptor for VEGF, flt-1, is also upregulated in tumour endothelial cells *in vivo*. The same and other authors recently reported (6,7) that both VEGF receptors, flk-1 and flt-1, are co-expressed in vascular cells in glioblastoma but not in low-grade glioma and that VEGF is present in significant amounts only in the vasculature of glioblastomas. Furthermore, a similar co-expression pattern of VEGF and its receptors has been reproduced in an animal model of human gliomas (17). The elevated production of VEGF in malignant gliomas versus low grade gliomas has been suggested to be due to the activation of epidermal growth factor receptor (EGF-r) in these tumours (18). We have also recently found that VEGF release is stimulated by growth factors and gangliosides, more prominently in malignant glioma cell lines (Koochekpour *et al*, submitted). In conclusion, although more cases should be investigated, quantitation of VEGF in cyst fluid of human gliomas may prove to be a valuable tool in the diagnosis of human gliomas.

Table I. Presence of VEGF in cyst fluid of human gliomas.

Patients	Grade	VEGF (µg/ml)
1	II	0.051
2	II	0.068
3	III	0.098
4	III	0.145
5	III	0.260
6	Glioblastoma multiforme	0.680
7	Glioblastoma multiforme	0.960

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